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The Barbara Ann Karmanos Cancer Institute (KCI) is dedicated to training young scientists for careers in research. The intent of this program is to broaden the number of students that can participate in KCI's undergraduate summer training endeavor by creating a focused effort utilizing the established Breast Cancer Program of our Comprehensive Cancer Center. It is our intent to recruit promising undergraduate science majors, give them the opportunity to take part in breast cancer research and impress them with the excitement of contributing to the cure/prevention of this dread disease. This summer research fellowship reflects KCI's conviction that elucidation of the biological basis of human cancer and the application of the results from basic research in the clinic requires knowledge and training in many disciplines including biochemmistry, pathology, molecular biology, immmunology, therapeutics, pharmacology and chemistry. The annual goal of this training program is to teach the techniques of bench research, develop within eight students the approach to critical scientific thought needed to pursue independent research and stimulate the student's desire for a future career in breast cancer research.

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Introduction

- During the summer of 2004, 8 outstanding undergraduate students from 7 universities were awarded fellowships for training in breast cancer research. Depending on the date the various universities completed their academic year, the trainees began their research experience in late May or early June of 2004 and completed 10 weeks of training in August.
- The 8 fellows were mentored by 7 individual faculty members (one faculty member mentored 2 fellows in his laboratory) during their training and conducted investigations in a variety of different topics concerned with breast cancer.
- Studies were carried out in genomic instability, death receptors, apoptosis, differential gene expression, purification of mutant ERα, metalloproteinase inhibition and development of breast cancer vaccines.
- During their training, fellows interacted not only with their mentor but had the opportunity to work with predoctoral students and postdoctoral fellows in their laboratory. Furthermore, the group of undergraduate fellows had the opportunity to discuss their research project with each other and attend institutional seminars and grand rounds, giving the trainees a feeling of the broad areas which cancer research encompasses.
- At the end of the training period on August 19, 2004, the fellows gave a poster presentation of their individual projects to the Karmanos Cancer Institute/Wayne State University faculty, students and postdoctoral fellows.

Body of Report

1. Recruiting

a. The color brochure describing the Undergraduate Summer Fellowships in Breast Cancer Research (see Appendix of progress report of March 31, 2003) was sent (October, 2003; 2nd mailing) to science departments of 75 colleges and universities in Michigan, Ohio, Indiana and Illinois. In addition, fellows from the summer of 2002 aided our recruiting efforts at their institutions. As a result, the program had 10 applications for the 8 positions in the summer of 2003. These efforts were more effective for the summer of 2004. There were 23 applications for the summer program in 2004 (Table I). These applicants were enrolled in 20 separate colleges and universities. Again, applications came from colleges and universities outside the Midwest (e.g. Maryland).

2. Initiation of Program

Last year (Summer 2004), we received 23 applications for the summer undergraduate fellowships. These applicants learned of the program by "word of mouth" and via our brochure which was distributed to 75 colleges and universities or through our website. Applicants sent letters indicating their interest in breast cancer research, their curriculum vitae and a description of their research accomplishments to date. Applications were screened by a 3-member recruiting committee and the materials for 8 top rated applicants were distributed to the 11 faculty of the training staff for

consideration. At a subsequent meeting of the faculty, the 8 fellows were matched with faculty having similar research interests.

The successful applicants were notified in April and fellowships starting dates arranged.

3. Training Program

The Fellows were assembled for an orientation on May 20, 2004 at which an overview of the program was presented and they were familiarized with laboratory and personnel procedures. At this time, most of the fellows met with their mentors. In spite of the variation in the academic year of the undergraduate universities of each fellow, start dates for the fellowships were kept to a narrow window. All trainees began their fellowship between May 24 and June 7.

The fellows selected for the program are listed in Table II along with their undergraduate universities, mentors and title of their research project (poster).

During the summer program, the progress of each student was monitored by the Director of the program. As each fellow reached the end of his/her 10-week training, instructions were given on preparation of posters with which to present their accomplishments. A "Poster Day" was held on August 19, 2004, during which the fellows presented their research to the body of fellows, the training faculty and the staff of the Karmanos Cancer Institute/Wayne State University. Each fellow was presented with a certificate denoting their successful participation in the program.

4. Evaluation of the Program and Modifications

We have found that by paying close attention to the progress of each fellow the training faculty can access the quality of the program. Students were routinely queried regarding what they expected from the experience and how this related to the training they were receiving. It appears that each found the fellowship beyond their expectations. This feeling persisted through the poster presentations.

In addition, faculty evaluation has been carried out after the training had been finished. Overall the mentors were excited about the students and their performance. It was felt that having the fellows begin their training within a narrow period of time benefited the Program. For example, the posters were of similar quality in demonstrating the student's accomplishment and understanding since all students had completed their projects by Poster Day

Follow-up questionnaires (attached with the previous progress report 2003) were sent again to the fellows. The fellows' responses enabled the training faculty to evaluate the fellowship after the students were able to reflect on their experience. To date, we have received responses from 19 of the 24 undergraduates who have completed the Program (2002-2004). We are pleased to learn that overall the fellows had a positive experience. All are impressed by their research experience and would recommend our program to other students.

This year the responses to the remaining questions in the questionnaire were all positive, a few fellows made specific comments which are of interest.

- "...found the summer research experience to have a positive influence on her academics."
- "...learned to be independent and how to research the background of a topic.
- "...the research experience had a strong influence on the interview for medical school."
- "...with this experience, I am more confident about going into research and graduate studies."
- "...now confident in ability to do independent research. Consequently, I have applied to graduate school (University of Pennsylvania)."

5. Reportable Outcomes

Two of the undergraduate breast cancer research fellows who completed their training with the support of DAMD17-02-1-0617 have been accepted for graduate training. Rachel Vonck will be matriculating into the University of Pennsylvania Graduate School (Immunology) this Fall and Elizabeth Masko matriculated in the Wayne State University's Graduate Program in Cancer Biology in September 2004.

Two of our fellows will be presenting their summer research at the Era of Hope meeting in Philadelphia, June 8-11, 2005. The title of Rachel Vonck's (summer of 2004) poster is "Difference in the kinetics and amplitude of ERK and FLIP (short) activation in highly homogeneous epitope-specific human CD8+ CTL cultures" (page 9). Chinyere Knight (summer of 2003) will present her work on "Removal of the ER-alpha F domain facilitates detection of difference in the activity of mutated receptors" (page 10).

Table I
Applicants to the 2004 Undergraduate Summer Fellowship in Breast Cancer Research

Students	College/Univ
Usman Afzal	Univ of Toledo
Keyunna Castleberry-Austin	Mi State
Alexandra Ciungu	Oakland Univ
Andrew Clark	Univ of Maryland
Elizabeth Dugan	MI State
Adam M. Forman	Univ of MI
Divya Gopalan	WSU
Patrick A. Hansma	Grand Valley St
Anthony Klemptner	Kalamazoo College
Nisha Jambulingam	Johns Hopkins
Miriam D. Kleinman	MSU
Brian M. Lin	John Hopkins
Patricia Mackin	Notre Dame
Jenifer Mayrberger	U of M-Flint
Jennifer Overbeck	Aquinas College
Lauren Raetz	Illinois Inst of Tech
Erin Scott	DePauw
Shivang P. Shah	Wabash College
Rachel K. Smitek	Miami Univ
Angela J. Soder	Ohio University
Emily Tromp	Purdue
Rachel Vonck	MI Tech Univ
Casey Vogelheim	Northerm Mi

Table II
Undergraduate Summer Fellows in Breast Cancer Research (2004)

Fellows	Undergraduate Institution	Mentor	Poster Title
1. Usman Afzal	Montana Tech	M. Shekhar, Ph.D.	Evaluation of the role of ubiquitin conjugating function in RAD6B-induced genomic instability.
2. Adam Forman	Univ. of Mich	K. Reddy, Ph.D.	The ability of Rottlerin to selectively up-regulate death receptors DR4 and DR5 in drug resistant cells.
3. Divya Gopalan	Wayne State Univ	A. Rishi, Ph.D.	Mechanisms of apoptosis signaling by CARP-1
4. M. Kleinman	Michigan St Univ.	A. Wali, Ph.D.	Differential gene expression patterns of NM23 among mesothelioma and breast cancer cell lines.
5. J. Mayrberger	Univ of Mich., Flint	D. Skafar, Ph.D.	Purification of wild-type and mutant hERα using a GSTrap FF.
6. E. Scott	DePauw Univ.	R. Fridman, Ph.D.	The role of synthetic matrix metalloproteinase inhibitors (MMPIs) on Pro-MMP-2 activation.
7. S. Shah	Wabash College	R. Fridman, Ph.D.	Matrix type metalloproteinase 17(MT-4MMP) in pcDNA3.1 and pTF7.
8. R. Vonck	Mich Tech Univ	J. Kan-Mitchell, Ph.D.	Investigation of mimic pepties based on protein tyrosine phosphorylation profile upon stimulation of T cell receptor.

DIFFERENCES IN THE KINETICS AND AMPLITUDE OF ERK AND FLIP(SHORT) ACTIVATION IN HIGHLY HOMOGENEOUS EPITOPE-SPECIFIC HUMAN CD8+ CTL CULTURES.

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Most cancer vaccine strategies have focused on induction of cytotoxic T lymphocytes (CTLs) that lyse tumor cells. As a result, a large number of HLA class I-restricted antigenic peptides from human tumor antigens have been identified to date. Candidate peptides used as therapeutic cancer vaccines in clinical trials, however, have failed to consistently elicit significant CTL responses in most patients immunized. In particular, CTL induction rarely associates with tumor regression, suggesting ineffectual T cell effector function. Therefore, improving our understanding of the mechanisms and molecular basis of the activation of CTLs may help to harness the exquisite specificity of the immune response to destroy cancer cells without affecting normal tissues.

Here we investigate whether human CTLs with different ligand specificities can exist in distinct activation states, as reflected by the kinetics and duration of ERK activation. Additionally, the susceptibility of CTLs with different specificities towards CD95-mediated apoptosis as measured by the level of expression of c-FLIP(short) is compared. Highly homogeneous (>95% tetramer+) peptide-specific CTLs were generated ex vivo from circulating CD8+ precursors of seronegative healthy donors. CTLs specific to the well-characterized, highly immunogenic CD4-independent HIV Gag p17 SL9 (SLYNTVATL) peptide are highly activated and sensitive to cytokine-induced apoptosis. In contrast, CTLs induced by an SL9 analog, p41 (SLYNTVAAL), and the p24 TV9 (TLNAWVKVV) have characteristics typical of stable effector cells. Using Western blot analysis, stimulation of SL9-CTLs by OKT3 cross-linking or cognate peptide-pulsed lymphoblastoid cells produced a more rapid and transient ERK phosphorylation when compared to p41- and TV9-CTLs. Moreover, SL9-CTLs expressed lower levels of FLIP(short) after stimulation.

Therefore, our results show that CTLs with different specificities can have distinctive consequences of antigen recognition in terms of the kinetics and amplitude of MAPK signaling and the modulation of FLIP(short). The character of these signaling proteins can modulate the outcome of TCR activation. These findings may provide new molecular measurements for what constitutes an "optimal" CTL response to peptide cancer vaccines. This line of research is perhaps most useful in the development of breast cancer vaccines, since peptide antigens from breast cancers cells have elicited T cells that are not particularly immunologically active.

The U.S. Army Medical Research and Materiel Command under DAMD17-02-1-0617 supported this work.

REMOVAL OF THE ER-ALPHA F DOMAIN FACILITATES DETECTION OF DIFFERENCES IN THE ACTIVITY OF MUTATED RECEPTORS.

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The human estrogen receptor-alpha (ERalpha) plays a key role in the development and progression of breast cancer, and responds to hormones and ligands in ways that are not completely understood. We have used structural information to identify a potential key interaction in the receptor, a potential hydrophobic interaction involving leucine-536 (L536) at the start of an important helix in the ligand-binding domain of the ER, helix 12. Substitution of this residue with either lysine (L536K) or asparagine (L536N) led to receptors with increased basal activity and eliminated E2-stimulated activity on an ERE-driven luciferase reporter in HeLa cells. This was surprising, since lysine possesses a large, positively-charged side chain, while that of asparagine is small and polar. Because the extreme carboxy-terminal region of the ER (the F domain) has been reported to stabilize the conformation of the ER, we wanted to test whether removing this domain would facilitate detection of differences in the activity of the lysine- and asparagine-substituted receptors. We therefore constructed double mutants in which not only L536 was mutated, but the F domain was deleted as well, L536K/S554stop and L536N/S554stop. We then analyzed the activity of these mutant ERs on an ERE-driven luciferase reporter in HeLa cells.

The basal activity of the L536K/S554stop mutant was no different than that of the wt ER, while the mutant exhibited a substantially lower response to E2 (2.5 ± 0.75-fold stimulation, compared with 7.3 ± 0.97-fold stimulation for the wt ER, n=3). The basal activity of the L536N/S554stop mutant was reduced by approximately half, while the mutant's response to E2 was eliminated (0.78 ± 0.16 -fold stimulation by E2, n=3).

These results provide further evidence that the F domain modulates the activity of the ERalpha. These results also show that removal of the F domain from the ER allows us to distinguish the effects of specific mutations on the activity of the ER. This will help us to determine the critical interactions that control the activity of the estrogen receptor in response to estrogen and tamoxifen, and so will assist in the design of better therapeutic and preventive agents for use in breast cancer.

The U.S. Army Medical Research and Materiel Command under DAMD17-02-1-0617 supported this work.